

Audrey P. Gasch · Margaret Werner-Washburne

The genomics of yeast responses to environmental stress and starvation

Received: 18 January 2002 / Accepted: 21 March 2002 / Published online: 30 April 2002
© Springer-Verlag 2002

Abstract Unicellular organisms such as yeast have evolved to survive constant fluctuations in their external surroundings by rapidly adapting their internal systems to meet the challenges of each new environment. One aspect of this cellular adaptation is the reorganization of genomic expression to the program required for growth in each environment. The reprogramming of genomic expression can be unveiled using DNA microarrays, which measure the relative transcript abundance of essentially every gene in an organism's genome. Characterizing environmentally triggered gene expression changes provides insights into when, where, and how each gene is expressed and offers a glimpse at the physiological response of the cells to changes in their surroundings. This review will focus on the genomic expression responses of the budding yeast *Saccharomyces cerevisiae* to diverse environmental changes, highlighting some of the themes that have emerged from the collection of published yeast genomic expression studies. The results of these studies present insights as to how yeast cells sense and respond to each new environment, and suggest mechanisms that this organism uses to survive stressful environmental changes.

Keywords *Saccharomyces cerevisiae* · Environmental sensing · Microarray · Whole-genome gene expression

Adversity has the effect of eliciting talents, which in prosperous circumstances would have lain dormant.

Horace

A.P. Gasch (✉)
Department of Genome Science,
Lawrence Berkeley National Laboratory,
1 Cyclotron Road 84-355, Berkeley, CA 94720, USA
e-mail: apgasch@lbl.gov
Tel.: +1-510-4866791, Fax: +1-415-2804084

M. Werner-Washburne
Department of Biology, University of New Mexico,
Albuquerque, NM 87131, USA

Life is stressful

A constant challenge for unicellular organisms in nature is maintaining internal homeostasis despite abrupt and dramatic fluctuations in the external conditions. Sudden changes in the external environment can perturb the internal system of the cells, disrupting cellular functions and preventing growth. Therefore, microorganisms must rapidly adapt to their surroundings by adjusting their internal milieu to function under the new conditions. Although cells growing under optimal conditions have been most carefully studied, the development of genomic analyses allows us to begin to understand the regulation and extent of responses under suboptimal conditions, the conditions that have shaped life over evolutionary time.

Yeast cells have evolved to survive sudden, often drastic and stressful changes in their environment. Some stressful conditions have been well studied experimentally, including growth at temperatures above or below around 25°C, growth in a medium of high osmolarity and ionic strength, exposure to toxic chemicals such as heavy metals, oxidizing agents, and DNA-damaging drugs, starvation for a variety of nutrients, irradiation, desiccation, and others. Many of these stressful conditions have been studied in the context of industrial uses of yeast, such as bread making and brewing, as these processes are affected by the response of cells to high temperatures, chilling, desiccation, and oxidation (Attfield 1997). As we improve our ability to study cellular responses to external signals, other environmental conditions that are stressful for yeast are likely to be identified, such as mixed cultivation with other microbes, exposure to microbial signaling molecules, or exposure to chemicals currently considered to be biologically inert.

In response to a sudden shift in conditions, yeast cells mount a multifaceted response that involves an often transient arrest of normal cellular processes during a period of reorganization of the internal milieu. Clearly, every level of cellular organization is involved in responding to environmental stresses. For example, in response to glucose starvation, there are cell surface proteins that

detect the absence of glucose and activate signal-transduction pathways in different cellular compartments (Johnston 1999; Estruch 2000; Igual and Estruch 2000), which govern changes in the phosphorylation, localization, and activity of proteins (Macfarlane et al. 1999; Vincent et al. 2001), changes in gene expression (DeRisi et al. 1997; Gasch et al. 2000) and translation (Fuge et al. 1994; Ashe et al. 2000; Kuhn et al. 2001), accumulation of stress-protectant molecules (Francois and Parrou 2001), and degradation of proteins and RNA (Albig and Decker 2001; Vasudevan and Peltz 2001). Thus, for the cell to respond effectively, changes in all of the cellular components must be completely integrated.

Global analyses

Although there is a great deal known about stress responses, there are many details that are not completely understood. The development of global analyses allows us to begin to understand the regulation, coordination, and extent of different aspects of the responses to sub-optimal conditions. DNA arrays can be used to assess a variety of cellular features, including transcript abundance, localization, and polysome association (Diehn et al. 2000; Takizawa et al. 2000; Kuhn et al. 2001), as well as the presence and location of DNA-binding proteins bound throughout the genome (Ren et al. 2000; Iyer et al. 2001; Lieb et al. 2001; Simon et al. 2001). The availability of the *S. cerevisiae* deletion set with tagged gene deletions (Ross-Macdonald et al. 1999; Winzeler et al. 1999) makes it possible to identify, among all the non-essential genes in yeast, those genes whose products are required for specific cellular processes (Chan et al. 2000; Bianchi et al. 2001; Birrell et al. 2001; Ni and Snyder 2001; Ooi et al. 2001) or contribute to stress resistance in a dose-dependent manner (Giaever et al. 1999). New approaches to the analysis of synthetic lethality (Tong et al. 2001), protein-protein interactions (Uetz et al. 2000; Ito et al. 2001; Gavin et al. 2002; Ho et al. 2002), global translation initiation (Blomberg 1995; Norbeck and Blomberg 1996; Godon et al. 1998; Lee et al. 1999; Appella et al. 2000), and protein abundance (Haab et al. 2001; Zhu and Snyder 2001; Zhu et al. 2001) increase the potential for a coordinated group of laboratories to drill through biological processes to develop a multidimensional, integrated view of how the cell works.

An important aspect of each cellular response to environmental change is the reorganization of gene expression. DNA arrays can be used to measure the relative transcript levels of essentially every gene in the yeast genome at any given moment, providing a glimpse of the genomic expression program. Exploring the dynamic nature of the yeast genome through time-course experiments can illuminate multiple aspects of the cellular response without the requirement of a priori knowledge. For example, hypothetical functions can be assigned to uncharacterized genes that are expressed similarly to well-studied genes, and regulatory mechanisms that gov-

ern gene co-expression can be inferred by identifying conserved sequence motifs, within and adjacent to the open reading frames, that may be involved in the regulation of gene expression (Eisen et al. 1998; Brown and Botstein 1999; Tavazoie et al. 1999; Hughes et al. 2000a; Bussemaker et al. 2001). Furthermore, a picture of the physiological state of cells can be constructed by considering the known functions of genes whose expression is affected under a given set of conditions, implicating cellular processes that mediate or respond to the observed gene expression changes.

This review will highlight recent advances in our understanding of the gene expression changes in the budding yeast *S. cerevisiae* as cells respond to environmental stress, focusing on trends that have emerged from the collection of published yeast genomic expression studies. Because each genome-scale experiment can produce so much information and because good datasets will inevitably be mined to produce new information for years to come, we wanted to provide a review of the literature and to discuss briefly what we see as the future directions and outcomes of this level of analysis. As more genomic expression data emerge, in combination with data from other whole-organism approaches, a dynamic picture of the integrated cellular response of yeast cells to environmental change can be constructed. This will allow researchers to address questions such as how modulation of stress responses affects fitness, the extent to which the responses to different stresses overlap, and whether different cell types have distinct responses to stress. Ultimately, the integration of genomic data with data obtained by more classical methods will lead to the ability not only to predict but also to modify and possibly construct novel responses in yeast and other organisms.

Genomic expression in response to environmental change

To date, a number of studies have been published that have examined gene expression in wild-type cells responding to environmental changes (Table 1). In most of these experiments, cells were shifted from standard growth conditions, i.e. 25–30°C in rich or defined medium, to environments thought to be stressful for cells, such as elevated temperatures, medium containing high concentrations of salt or sorbitol, acidic or alkaline surroundings, and drugs that perturb the cellular redox potential, damage cellular structures, and inhibit enzymatic activities. In most cases, when cells were shifted to these environments, they responded with changes in the expression of hundreds or thousands of genes, revealing the plasticity of genomic expression. Comparing the genomic expression programs elicited by different environmental transitions reveals that some of the expression changes are specific to each new environment, while others occur in all of the experiments tested and represent a common response to environmental stress. Features

Table 1 Genomic expression studies characterizing wild-type yeast responses to stress

Environment	References
Heat shocks	(Gasch et al. 2000; Causton et al. 2001)
Ethanol shock	(Alexandre et al. 2001)
pH extremes	
Acid	(Causton et al. 2001; Kapteyn et al. 2001)
Alkali	(Causton et al. 2001)
Oxidative and reductive stress	
Hydrogen peroxide	(Gasch et al. 2000; Causton et al. 2001)
Menadione	(Gasch et al. 2000)
Diamide	(Gasch et al. 2000)
Cadmium	(Momose and Iwahashi 2001)
DTT	(Gasch et al. 2000; Travers et al. 2000)
Hyper-osmotic shock	
Sorbitol	(Gasch et al. 2000; Rep et al. 2000; Causton et al. 2001)
Potassium/sodium chloride	(Posas et al. 2000; Rep et al. 2000; Causton et al. 2001; Yale and Bohnert 2001; S.M. O'Rourke and I. Herskowitz, personal communication)
Starvation	
Progression into stationary phase	(DeRisi et al. 1997; Gasch et al. 2000)
Amino acid starvation	(Gasch et al. 2000; Jia et al. 2000; Natarajan et al. 2001)
Nitrogen starvation	(Gasch et al. 2000)
Phosphate starvation	(Ogawa et al. 2000)
Zinc starvation	(Lyons et al. 2000)
Copper starvation	(Gross et al. 2000)
Respiration	
Petite mutants	(Traven et al. 2001)
Non-fermentable carbon sources	(Kuhn et al. 2001)
Anaerobic growth	(Kwast et al. 2002)
Sporulation	(Chu et al. 1998; Primig et al. 2000)
Diverse drug treatments	(Bammert and Fostel 2000; Hughes et al. 2000b)
Exposure to alpha factor	(Spellman et al. 1998; Roberts et al. 2000)
DNA damaging agents	
Alkylating agents	(Jelinsky and Samson 1999; Jelinsky et al. 2000; Gasch et al. 2001; Natarajan et al. 2001)
Ionizing radiation	(De Sanctis et al. 2001; Gasch et al. 2001)
Double-strand breaks	(Lee et al. 2001)

of the common and specific gene expression changes are discussed below.

The environmental stress response: a common response to stressful environments

Overall, each genomic expression program is unique to the features of the environment (see below), however comparative analysis of the genomic expression responses to diverse environmental changes revealed that the expression of roughly 900 genes (around 14% of the total number of yeast genes) is stereotypically altered following stressful environmental transitions (Gasch et al. 2000). A similar set of genes was also identified in a related study (Causton et al. 2001), further indicating the commonality of these gene expression changes. The genes that participate in this response, referred to here as the environmental stress response (ESR), fall into two groups based on their expression patterns: one group is

composed of around 600 genes whose transcripts are decreased in abundance following stressful environmental transitions (referred to as repressed genes), and a second group is composed of around 300 genes whose transcripts increase in abundance in response to the transitions (referred to as induced genes; reviewed in Gasch 2002). The genes in these two groups display nearly identical but opposite changes in gene expression in response to essentially all of the conditions tested, suggesting that they are components of the same cellular response.

The ESR is initiated in response to many different stressful environments in a manner that is sensitive to the degree of cellular stress. When cells are shifted from optimal growth conditions to conditions thought to be stressful for the cell, they respond with changes in ESR gene expression that are proportional to the magnitude of environmental change. This correlation was identified through a series of dosage experiments, in which cells experiencing a more severe environmental transition re-

sponded with larger changes in ESR gene expression relative to cells exposed to a more subtle environmental change (Gasch et al. 2000; Jelinsky et al. 2000). In contrast to sub-optimal transitions, when cells that have been adapted to alternate environments are returned to standard growth conditions, they respond with reciprocal changes in the expression of the ESR genes, indicating that the response is rapidly alleviated when cells are shifted to more optimal conditions (Gasch et al. 2000). Thus, the ESR is initiated in response to stressful environments, in proportion to the degree of stress experienced by the cell, making the magnitude of ESR gene expression changes a useful experimental gauge of the cellular stress level.

Based on the sensitivity of the ESR as well as the functional roles of the characterized genes in the response, it was proposed that the ESR protects critical functions within the cell during times of stress (Gasch et al. 2000). More than 70% of the characterized genes whose expression is repressed as part of the ESR are involved in protein synthesis (Ashburner et al. 2000; Ball et al. 2000), including genes required for ribosome synthesis and processing, RNA polymerase I- and III-dependent transcription, and protein translation; many of the uncharacterized genes in this group are likely to be functionally related. The reduced synthesis of these transcripts and their products may help to conserve energy while the cell adapts to its new conditions, a role that has been previously proposed for the reduced expression of genes encoding ribosomal proteins (Warner 1999). In contrast to these functionally related genes, the genes whose expression is induced in the ESR are involved in a wide variety of cellular processes, including carbohydrate metabolism, protein folding and degradation, oxidative stress defense, autophagy, cytoskeletal reorganization, DNA-damage repair, and other processes. The functions of these gene products may protect critical aspects of the internal milieu, such as energy reserves, the balance of the internal osmolarity and oxidation-reduction potential, and the integrity of cellular structures including proteins and DNA. The protection of these features by the ESR gene products likely contributes to the cross-resistance of yeast cells to multiple stresses, in which cells exposed to a mild dose of one stress become tolerant of an otherwise-lethal dose of a second stressful condition (Mitchel and Morrison 1982; Blomberg et al. 1988; Wieser et al. 1991; Flattery-O'Brien et al. 1993; Schüller et al. 1994; Lewis et al. 1995).

Although initiation of the ESR is a common response to stressful environments, regulation of ESR gene expression is condition-specific and is controlled at multiple levels, including transcription initiation, silencing through chromatin remodeling, and regulated mRNA turnover (reviewed in Gasch 2002). For example, several of the transcription factors that control ESR gene expression are active only under specific conditions: the transcription factors Hsf1p, Hot1p, and Yap1p independently affect the expression of subsets of ESR genes in response to heat shock, osmotic shock, or oxidative stress,

respectively, but are uninvolved in regulating the expression of these ESR genes under other conditions (Treger et al. 1998; Rep et al. 1999, 2000; Gasch et al. 2000; Amoros and Estruch 2001). The so-called "general stress" transcription factors Msn2p and Msn4p have been implicated in regulating many of the induced ESR genes (Gasch et al. 2000; Causton et al. 2001), although the role of these factors also varies under different conditions (Treger et al. 1998; Rep et al. 1999, 2000; Garreau et al. 2000; Gasch et al. 2000; Amoros and Estruch 2001). A number of condition-specific signaling pathways have been implicated in mediating the coordinated expression of the induced and repressed ESR genes, including the protein kinase C MAP kinase pathway following secretion defects and cell wall damage (Jung and Levin 1999; Nierras and Warner 1999; Li et al. 2000; A.P. Gasch and P.O. Brown, unpublished data), the MEC1 pathway following DNA damage (Gasch et al. 2001), and the Ssk1p/Stell1p-dependent pathways and the MAP kinase Hog1p in response to osmotic stress (Posas et al. 2000; Rep et al. 2000; S.M. O'Rourke and I. Herskowitz, personal communication). Pathways that suppress the ESR, including the TOR and protein kinase A pathways, have also been implicated, although the conditions under which these signaling networks govern ESR gene expression have not been defined (Marchler et al. 1993; Klein and Struhl 1994; Neuman-Silberberg et al. 1995; Gerner et al. 1998; Thevelein and de Winder 1999; Norbeck and Blomberg 2000; Thevelein et al. 2000; Barbet et al. 1996; Beck and Hall 1999; Powers and Walter 1999). Each of the pathways that control ESR gene expression is known to be activated by specific cellular signals, and consistently, most of the pathways tested govern ESR initiation only under the conditions that trigger their activity (Nieras and Warner 1999; Gasch et al. 2001). Thus, despite the commonality of ESR initiation, the program is regulated by condition-specific mechanisms, allowing the cell to activate the ESR in response to a wide variety of upstream signals while maintaining specificity in how the cell senses and otherwise responds to each new environment.

Specificity of genomic expression programs

The precision with which the cell responds to each environment is evident in the differences in the genes whose expression is affected, as well as the magnitude and dynamics of this expression. In addition to triggering ESR initiation, many of the environmental transitions provoke expression changes in highly specialized genes. For example, in response to conditions that damage DNA, the cell responds in part by inducing a small subset of genes that are specifically enlisted in the cellular response to the damage, presumably to aid in the repair of the genome (Jelinsky and Samson 1999; Jelinsky et al. 2000; Gasch et al. 2001; Lee et al. 2001). When zinc, copper, or phosphate are depleted from the medium, the cells increase the expression of genes encoding high affinity

transporters of these compounds to scavenge the limited nutrients, while inducing genes that mobilize intracellular zinc, copper, or phosphate (Gross et al. 2000; Lyons et al. 2000; Ogawa et al. 2000). In contrast, depletion of external amino acids stimulates the repression of genes encoding specific amino acid transporters and induction of genes encoding general-specificity transporters (Gasch et al. 2000); amino acid starvation also leads to the induction of genes that encode amino acid biosynthetic enzymes (Gasch et al. 2000; Jia et al. 2000; Natarajan et al. 2001).

Genomic expression programs triggered by environmental changes also exhibit distinctions in the kinetics of each response. Some environmental transitions provoke immediate reaction of the cells, with gene expression changes occurring within minutes of the transition. Heat shock, for example, which can rapidly denature proteins, results in changes in gene expression within minutes of the shock (Werner-Washburne et al. 1989; Gasch et al. 2000; Causton et al. 2001). In contrast, in response to drugs that prevent proper secretion of proteins through the endoplasmic reticulum (ER), the expression of a small number of genes (involved in the response to unfolded proteins in the ER) is induced early, but the majority of observed gene expression changes occur hours after drug exposure, perhaps due to secondary effects of the drugs (Gasch et al. 2000; Travers et al. 2000). Different levels of the same stress can also cause differences in response kinetics: cells exposed to a severe dose of sodium chloride (0.8 M) respond with delayed timing compared to cells exposed to a low salt concentration (0.4 M), for reasons that are not understood (Posas et al. 2000). Furthermore, the rate of each response can be affected by the state of the cells before the environmental transition (Cheng and Yang 1996; Siderius et al. 1997, 2000). These examples underscore the value of time-course experiments and, potentially, the study of these responses during different physiological or developmental states for complete understanding of cellular responses to a particular stress.

Many of the environmental changes studied to date present pleiotropic challenges for the cell. Comparison of the resulting genomic expression programs suggests that cells respond independently to each feature of the new environment to provide a composite genomic expression program unique to the combined characteristics of the new conditions. When cells are exposed to two, simultaneous environmental changes, for example a mild temperature shift combined with hypo-osmotic shock, the resulting genomic expression program largely approximates the sum of the gene expression responses to each individual transition (Gasch et al. 2000). This suggests that the cell responds independently to the effects of temperature shift and to the challenges of hypo-osmotic shock. Another example of a composite genomic expression program is the response to the methylating agent methylmethane sulfonate (MMS), which methylates DNA, proteins, glutathione, and likely other cellular components (Paik et al. 1984; Mizumoto et al. 1993;

Wilhelm et al. 1997). Cells exposed to MMS induce the expression of a number of genes specific to DNA damage, but they also trigger expression changes in genes involved in protein folding and degradation, oxidative stress defense, amino acid metabolism, and aerobic processes (Jelinsky and Samson 1999; Jelinsky et al. 2000; Gasch et al. 2001; Natarajan et al. 2001) through signaling networks that are distinct from the DNA-damage specific signaling pathway (Gasch et al. 2001). These examples suggest that the cell regulates its genome with multiple, independent regulatory systems to orchestrate a precise genomic expression program that is customized to each environment.

Genomic analyses of starvation/stationary phase

Starvation for nutrients may be the most common stress experienced by microorganisms, yet the literature in this area still does not provide a complete understanding of the cellular response to starvation and re-feeding. Immediate responses to starvation have been relatively amenable to both genetic and molecular analyses, but longer-term responses to starvation have been more intractable, primarily because metabolic rates and physiological responses are slower in these cells. Very few mutants are known that lose viability after long-term starvation and none of the genes described as "essential" by the Yeast Deletion Consortium (Winzeler et al. 1999) is required for long-term survival, i.e. after 7 or more days in culture.

Starvation is a complex, albeit common, stress for microorganisms. The nutrients for which a cell can be starved include carbon and nitrogen, with other elements such as phosphate, sulfur, and metals being less commonly evaluated. Starvation for specific nutrients provides cues for developmental responses throughout the fungi, e.g. nitrogen starvation is frequently required for mating or sexual development (Nelson and Metzenberg 1992; Alspaugh et al. 1997; Wang et al. 2000). Starvation for carbon is the cue for haploid or diploid yeast cultures to enter stationary phase (Werner-Washburne et al. 1993, 1996) and, under certain conditions, for haploid yeast cells to grow invasively (Cullen and Sprague 2000). In the presence of a poor carbon source, starvation for nitrogen induces sporulation and in the presence of a good carbon source stimulates pseudohyphal growth (Pan et al. 2000; Zaragoza and Gancedo 2000). One question in the study of starvation responses is whether all starvations lead to the same long-term physiological state. Earlier work has led to the conclusion that cells starved of nitrogen do not enter a quiescent state (Granot and Snyder 1993), but the basis for the difference between arrests induced by carbon starvation versus nitrogen starvation is not known. We anticipate that genomic analysis will change this.

The process of entry into stationary phase, best studied in rich medium (2% glucose) at 30°C, exhibits several distinct phases. When quiescent cells are transferred to

fresh medium, there is a lag phase as they adjust to the new growth conditions and exit the quiescent state. This is followed by the exponential phase, during which growth rates are maximal. When glucose is depleted from the medium, cultures go through the diauxic shift as cells transiently arrest growth and reorganize their metabolism in order to obtain energy from non-fermentable carbon sources, such as ethanol and acetate. Once cell division is reinitiated, cultures enter the post-diauxic phase, a period of slow growth that can last days, during which time the culture density doubles. In this phase, growth is supported by non-fermentable carbon sources. Cell cultures enter stationary phase between 5 and 7 days after inoculation, when carbon sources are exhausted and culture densities cease to increase (Werner-Washburne et al. 1993, 1996). Each of these stages is marked by different changes in gene expression. At the diauxic shift transition, cells induce the expression of thousands of genes, including those encoding proteins involved in respiration, fatty acid metabolism, and the glyoxylate cycle, while initiating the ESR (DeRisi et al. 1997; Gasch et al. 2000; M. Werner-Washburne, unpublished data). Most of these expression differences persist through the post-diauxic phase, but many subside slightly as the cells enter stationary phase. A large number of gene expression changes occur late in stationary phase, including the reduced expression of many genes involved in secretion, membrane and cell wall synthesis, amino acid metabolism, cell-cycle progression, and other processes required for growth and division. A subset of genes is induced late in stationary phase (including *SNZ1*), indicating that gene expression changes occur late in response to nutrient starvation and may be important to cell survival under these conditions (Braun et al. 1996; Padilla et al. 1998). As a result of genomic analyses, many more co-expressed genes have now been identified, contributing to our understanding of the processes that occur during each phase of the progression to stationary phase.

Another prevalent stress in nature is nitrogen starvation. Gasch and colleagues have published 32 microarray experiments following the response of cells to long-term nitrogen starvation in synthetic medium and progression to stationary phase in rich medium, over a period of 5 days (Gasch et al. 2000). The number of time points and the availability of two sets of data from entry into stationary phase in the SGD Expression Connection (<http://genome-www4.stanford.edu/cgi-bin/SGD/expression/expressionConnection.pl>) make this dataset extremely important for beginning to understand the dynamics of this process in budding yeast.

In examining this data, it is evident that many of the gene expression changes are distinctly different in response to nitrogen starvation compared with entry into stationary phase in rich medium. When cells were shifted to synthetic medium with limited nitrogen sources (and lacking amino acids), they rapidly induced the expression of genes involved in amino acid biosynthesis and allantoin utilization and initiated the ESR; within a few hours, the external nitrogen sources were depleted,

and the expression of many genes was induced, including those encoding proteins involved in respiration and carbon metabolism, sporulation, pseudohyphal growth, as well as many uncharacterized gene products (Gasch et al. 2000). Many of the gene expression changes that occurred late in the time course, during which time glucose became exhausted from the medium, were similar to those seen for cells approaching stationary phase, suggesting that the nitrogen-starved cells eventually became starved for carbon and that some of the gene expression differences may have been due to the combined effects of carbon and nitrogen starvation (C.M. Kao, personal communication).

Because nitrogen-starved cells were grown in synthetic medium and stationary-phase cultures were grown in rich medium, some of differences in gene expression under the two conditions may be the result of differences in the media. However, other differences are likely to be due to the cells' specific responses to carbon versus nitrogen starvation. Identifying those genes whose change in expression is specifically due to each starvation regime is critical to identifying the different switches triggered by long-term nitrogen versus carbon starvation. Identifying those genes whose expression changes as a result of incubation in synthetic medium as compared with rich medium will also yield important information, because cells in synthetic complete medium are unable to survive for long periods of time when grown in continuous culture, i.e. to growth arrest (M. Werner-Washburne, unpublished data). If the corresponding experiments for growth of wild-type cells in synthetic complete medium were available, it would be possible to determine those genes that were affected specifically by nitrogen or carbon limitation or by growth in synthetic complete medium.

An interesting question is whether quiescent yeast cells are actually able to mount a response at the level of gene expression. One study following the response of starved cells to MMS approached this question (Jelinsky et al. 2000). A 3-day-old culture of cells, likely in the post-diauxic phase of growth, was exposed to MMS. Many of the genes that are induced in exponential-phase cells responding to MMS were already highly expressed in the 3-day-old cells, even in the absence of the drug. Nonetheless, the expression of these and more genes was further induced after MMS treatment, indicating that these starved cells were still capable of mounting a gene expression response. In our laboratory, we have observed that stationary-phase cultures can respond to increasing levels of sodium chloride by altering gene expression, further indicating that these cells are capable of initiating a gene expression response to additional stress (M. Werner-Washburne, unpublished data).

Yeast starvation experiments, especially those involving long-term starvation, require some caution. For example, baseline data for long-term starvation is best obtained using prototrophic cells in order to avoid confusion from changes that are specific to auxotrophic strains. Addition of exogenous auxotrophic requirements

does not guarantee that internal concentrations are identical to concentrations in prototrophs throughout the culture cycle. Secondly, it has been known for 20 years that mRNAs isolated from cells in stationary-phase (probably post-diauxic) cultures contained little or no poly(A) mRNA (Sogin and Saunders 1980). To our knowledge, this experiment has not been repeated, especially in cells from 5- to 7-day-old cultures grown in YPD. We know the poly(A) tail length probably doesn't affect translation in stationary phase because mRNA present in quiescent cells has been shown to be translated (Fuge et al. 1994) and poly(A) tails have recently been reported to be dispensable for translation (Searfoss and Wickner 2000). However, poly(A) tail length becomes critical when using assays based on isolation of poly(A) mRNA or using microarray probe labeling techniques requiring poly(A) tails. In these cases, differences in poly(A) tail length may increase the variability of the assay, especially in very old cultures.

Adaptation of genomic expression following stressful environmental changes

A recurring feature of the genomic expression responses to environmental change is that most of these responses, with the exception of starvation responses, are transient (Powers and Walter 1999; Rep et al. 1999; Gasch et al. 2000; Jelinsky et al. 2000; Jia et al. 2000; Causton et al. 2001; Yale and Bohnert 2001). After a shift to a stressful environment, cells respond with large changes in gene expression; however, over time the gene expression differences often subside, and transcript levels adjust to levels closer to those seen in unstressed cells, even in the continued presence of the stress. This transient period of large gene expression changes likely represents an adaptation phase, during which time the cell adjusts its internal system to that required to function under the new conditions. Consistent with this model is the observation that there is a direct correlation between the magnitude of the environmental shift and the duration of the adaptation phase, suggesting that more time is required for cells to adapt to a severe environmental transition (Gasch et al. 2000).

Not all environmental stress responses result in transient gene expression changes. Under conditions that do not permit cellular growth, such as carbon starvation, cells enter a quiescent state to await a transition to nutrient-replete surroundings (Werner-Washburne et al. 1993, 1996). In this situation, the gene expression changes that occur as nutrients run out are not transient but rather persist for long periods of time (Braun et al. 1996; Gasch et al. 2000; M. Werner-Washburne, unpublished data). Not all of the genes induced during entry into stationary phase are required for survival, but it is assumed that they are important for this phase of the life cycle. What is known, however, is that the pattern of translated proteins in these cells is essentially the same as patterns of translated proteins in dividing, respiring cells, despite the

differences in gene expression (Fuge et al. 1994). Thus, for quiescent cells, translational regulation is likely to be an important level of control of protein synthesis.

Stress relief

Surviving sudden changes in environmental conditions is critical for survival, but equally important is the ability to resume activity once the cell has adapted to the new environment or has been moved to more favorable surroundings. This aspect of the response to environmental changes has not been well studied.

It has been observed that when cells growing at sub-optimal conditions, for example elevated temperatures or at high osmolarity, are returned to more optimal conditions, most transcript levels rapidly adjust to the steady-state program required for growth in optimal conditions (Gasch et al. 2000). In particular, the ESR is rapidly alleviated with no observable transient changes in gene expression. This suggests that dividing cells can readily adapt to optimal conditions and for the most part do not require the large, transient changes in gene expression seen when cells are shifted to sub-optimal environments.

In contrast, when quiescent, starved cells are provided with nutrients, they respond with large, transient changes in gene expression within 15 min of re-feeding, i.e. 10- to 40-fold for some genes (M. Werner-Washburne, unpublished data). While there is a set of genes whose expression decreases as cells exit stationary phase, one of the first groups of genes to be induced is a cohort of genes (almost 300) that include essentially all of the ribosomal protein genes in yeast. Gene expression changes during exit from stationary phase are dynamic and involve a large portion of the genome. This transition requires quiescent cells to resume cell-cycle progression and division, and the complex genomic expression program likely reflects that this transition is more complicated developmentally than shifting dividing cells between different environments.

Experimental design in exploring genomic expression responses to stress

A number of different experimental approaches have been used to study gene expression in cells responding to different environments, and each method presents different features of stress responses. The temporal patterns of gene expression changes identified through time-course experiments can be useful for identifying different phases of a given response (including the distinct phases of starvation, as discussed above) or distinguishing primary versus secondary responses to a given stimulus that may show distinct kinetics. Another approach is to use complementary single time-point measurements to identify specific aspects of a given response. For example, Lyons et al. (2000) compared gene expression in wild-type cells and cells lacking the zinc-responsive transcription factor

Zap1p as the cells responded to zinc depletion. The authors were able to identify 111 genes that responded to zinc deficiency in a Zap1p-affected manner: 46 of these genes contained the known Zap1p-binding site, while the remaining genes may contain a distinct Zap1p-recognized binding site or may be regulated by a second, yet unidentified factor (Lyons et al. 2000). A similar experimental design was undertaken by Ogawa et al. (2000) to identify genes whose expression was regulated by phosphate abundance: 22 genes whose expression was affected by low phosphate and dependent on known phosphate-sensitive regulators were identified, and the involvement of the identified gene products in polyphosphate synthesis and utilization revealed the importance of this metabolite in the response to phosphate starvation.

Another useful approach is to characterize the dose-dependent effects of a given stimulus. The magnitude of gene expression changes is proportionate to the magnitude of the environmental change, as observed in a number of studies (Gasch et al. 2000; Jelinsky et al. 2000; Posas et al. 2000). Furthermore, large doses of stress typically lead to more detectable changes in gene expression, perhaps because secondary effects that arise from severe stress trigger additional expression differences. In addition to the number and magnitude of gene expression changes, the timing of each response can also be affected by the amount of stress (Posas et al. 2000). These observations underscore the value of dosage studies when studying the response to stressful stimuli.

Advances, challenges, and future directions

The genomic expression studies mentioned here have contributed to our understanding of yeast responses to environmental diversity at a number of levels. Hypothetical functions have been suggested for many of the uncharacterized yeast genes based on their expression patterns, and a number of those hypotheses have been borne out through experimentation (Gachotte et al. 2001; Liu and Thiele 2001; Rabitsch et al. 2001; Valencia et al. 2001). Many of the studies mentioned here characterized gene expression not only in wild-type cells but also in mutant strains defective in transcription factors (Casagrande et al. 2000; Gasch et al. 2000; Gross et al. 2000; Jelinsky et al. 2000; Lyons et al. 2000; Ogawa et al. 2000; Rep et al. 2000; Travers et al. 2000; Causton et al. 2001), general repressors and chromatin remodeling factors (DeRisi et al. 1997; Fazzio et al. 2001), and signaling pathways and cell sensors (Jung and Levin 1999; Li et al. 2000; Posas et al. 2000; Rep et al. 2000; Roberts et al. 2000; Gasch et al. 2001) that have been implicated in each response. In fact, in some cases the regulatory factors involved in a response (for example, Rpn4p in the response to MMS) were initially implicated by the gene expression program in wild-type cells, based on the presence of the known binding sites of regulatory factors

in the promoters of coexpressed genes (Jelinsky et al. 2000; Gasch et al. 2000). On a more global scale, mechanisms that yeast cells use to survive stressful environments have been inferred from the published set of gene expression studies. These mechanisms include specific gene expression changes that counteract the challenges of each environment as well as the ESR, which may generally protect the cell in response to diverse environmental insults.

There is still a great deal to learn about stress responses at the level of gene expression. Time-course experiments, based on careful experimental design, will eventually allow us to distinguish the individual responses to each environmental feature, an important consideration given the pleiotropic nature of most environmental changes. To date, most published microarray studies have characterized gene expression in populations of asynchronous cells, and an important question is how the genomic expression programs differ in synchronized cultures and individual cells. Along this line, it was recently shown that the genomic expression response to DNA-damaging agents is different in cells synchronized at different points in the cell-cycle (Jelinsky et al. 2000). Furthermore, that individual cells in a population show differential resistance to a given stressful condition hints that there may be cell-specific differences in the responses, including gene expression changes (Sumner and Avery 2002). Finally, we do not know the ability of quiescent cells to respond to additional stresses, and although there is some indication that these cells can mount additional gene expression responses, the extent and dynamics of these responses are not yet known.

Genomic expression studies performed to date have provided a starting point for understanding the mechanisms that yeast use to survive some of the environmental conditions that cells experience in the wild. Characterization of the individual genes whose expression is affected under different situations will help us to better understand the roles served by their gene products. Integrating genomic expression data with information about global changes in protein synthesis and metabolites, as well as information gained from more classical methods, will provide a more complete picture of the cellular adaptation to environmental variation. This information will not only contribute to our understanding of yeast responses to stress in natural environments, but can also be applied to commercial uses of this organism (Attfield 1997). Finally, a better understanding of yeast responses to environmental stress will contribute to other areas of research, for example understanding the connection between stress and aging, mapping signal transduction pathways, identifying novel antibiotics, and exploring the evolution and adaptive significance of stress responses in yeast and other organisms.

Acknowledgements Special thanks to Sean O'Rourke and Ira Herskowitz for sharing data before publication, and Stefan Hohmann and Cammy Kao for helpful discussions. A.P.G. was funded by the National Science Foundation Postdoctoral Fellowship in Biological Informatics through the U.S. Department of Energy under No. ED-AC03-76SF00098. M.W.W. was funded by grants from NSF (MCB-0092374), NIH (R21-HG02262) and by Laboratory Directed Research and Development, Sandia National Laboratories, U.S. Department of Energy (DE-AC04-94AL85000).

References

- Albig A, Decker C (2001) The target of rapamycin signaling pathway regulates mRNA turnover in the yeast *Saccharomyces cerevisiae*. *Mol Biol Cell* 12:3428–3438
- Alexandre H, Ansanay-Galeote V, Dequin S, Blondin B (2001) Global gene expression during short-term ethanol stress in *Saccharomyces cerevisiae*. *FEBS Lett* 498:98–103
- Alspaugh JA, Perfect JR, Heitman J (1997) *Cryptococcus neoformans* mating and virulence are regulated by the G-protein alpha subunit GPA1 and cAMP. *Genes Dev* 11:3206–3217
- Amoros M, Estruch F (2001) Hsf1p and Msn2/4p cooperate in the expression of *Saccharomyces cerevisiae* genes HSP26 and HSP104 in a gene- and stress type-dependent manner. *Mol Microbiol* 39:1523–1532
- Appella E, Arnott D, Sakaguchi K, Wirth PJ (2000) Proteome mapping by two-dimensional polyacrylamide gel electrophoresis in combination with mass spectrometric protein sequence analysis. *Exs* 88:1–27
- Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 25:25–29
- Ashe MP, De Long SK, Sachs AB (2000) Glucose depletion rapidly inhibits translation initiation in yeast. *Mol Biol Cell* 11:833–848
- Attfield PV (1997) Stress tolerance: the key to effective strains of industrial baker's yeast. *Nat Biotechnol* 15:1351–1357
- Ball CA, Dolinski K, Dwight SS, Harris MA, Issel-Tarver L, Kasarskis A, Scafe CR, Sherlock G, Binkley G, Jin H, Kaloper M, Orr SD, Schroeder M, Weng S, Zhu Y, Botstein D, Cherry JM (2000) Integrating functional genomic information into the *Saccharomyces* genome database. *Nucleic Acids Res* 28:77–80
- Bammert GF, Fostel JM (2000) Genome-wide expression patterns in *Saccharomyces cerevisiae*: comparison of drug treatments and genetic alterations affecting biosynthesis of ergosterol. *Antimicrob Agents Chemother* 44:1255–1265
- Barbet NC, Schneider U, Helliwell SB, Stansfield I, Tuite MF, Hall MN (1996) TOR controls translation initiation and early G1 progression in yeast. *Mol Biol Cell* 7:25–42
- Beck T, Hall MN (1999) The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature* 402:689–692
- Bianchi MM, Ngo S, Vandenbol M, Sartori G, Morlupi A, Ricci C, Stefani S, Morlino GB, Hilger F, Carignani G, Slonimski PP, Frontali L (2001) Large-scale phenotypic analysis reveals identical contributions to cell functions of known and unknown yeast genes. *Yeast* 18:1397–1412
- Birrell GW, Giaever G, Chu AM, Davis RW, Brown JM (2001) A genome-wide screen in *Saccharomyces cerevisiae* for genes affecting UV radiation sensitivity. *Proc Natl Acad Sci USA* 98:12608–12613
- Blomberg A (1995) Global changes in protein synthesis during adaptation of the yeast *Saccharomyces cerevisiae* to 0.7 M NaCl. *J Bacteriol* 177:3563–3572
- Blomberg A, Larsson C, Gustafsson L (1988) Microcalorimetric monitoring of growth of *Saccharomyces cerevisiae*: osmotolerance in relation to physiological state. *J Bacteriol* 170:4562–4568
- Braun EL, Fuge EK, Padilla PA, Werner-Washburne M (1996) A stationary-phase gene in *Saccharomyces cerevisiae* is a member of a novel, highly conserved gene family. *J Bacteriol* 178:6865–6872
- Brown PO, Botstein D (1999) Exploring the new world of the genome with DNA microarrays. *Nat Genet* 21:33–37
- Bussemaker HJ, Li H, Siggia ED (2001) Regulatory element detection using correlation with expression. *Nat Genet* 27:167–171
- Casagrande R, Stern P, Diehn M, Shamu C, Osario M, Zuniga M, Brown PO, Ploegh H (2000) Degradation of proteins from the ER of *S. cerevisiae* requires an intact unfolded protein response pathway. *Mol Cell* 5:729–735
- Causton HC, Ren B, Koh SS, Harbison CT, Kanin E, Jennings EG, Lee TI, True HL, Lander ES, Young RA (2001) Remodeling of yeast genome expression in response to environmental changes. *Mol Biol Cell* 12:323–337
- Chan TF, Carvalho J, Riles L, Zheng XFS (2000) A chemical genomics approach toward understanding the global functions of the target of rapamycin protein (TOR). *Proc Natl Acad Sci USA* 97:13227–13232
- Cheng CY, Yang ST (1996) Dynamics and modeling of temperature-regulated gene product expression in recombinant yeast fermentation. *Biotechnol Bioeng* 50:663–674
- Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, Brown PO, Herskowitz I (1998) The transcriptional program of sporulation in budding yeast. *Science* 282:699–705
- Cullen PJ, Sprague GF (2000) Glucose depletion causes haploid invasive growth in yeast. *Proc Natl Acad Sci USA* 97:13619–13624
- DeRisi JL, Iyer VR, Brown PO (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278:680–686
- De Sanctis V, Bertozzi C, Costanzo G, Di Mauro E, Negri R (2001) Cell cycle arrest determines the intensity of the global transcriptional response of *Saccharomyces cerevisiae* to ionizing radiation. *Radiat Res* 156:379–387
- Diehn M, Eisen MB, Botstein D, Brown PO (2000) Large-scale identification of secreted and membrane-associated gene products using DNA microarrays. *Nat Genet* 25:58–62
- Eisen MB, Spellman PT, Brown PO, Botstein D (1998) Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 95:14863–14868
- Estruch F (2000) Stress-controlled transcription factors; stress-induced genes and stress tolerance in budding yeast. *FEMS Microbiol Rev* 24:469–486
- Fazzio TG, Kooperberg C, Goldmark JP, Neal C, Basom R, Delrow J, Tsukiyama T (2001) Widespread collaboration of Isw2 and Sin3-Rpd3 chromatin remodeling complexes in transcriptional repression. *Mol Cell Biol* 21:6450–6460
- Flattery-O'Brien J, Collinson LP, Dawes IW (1993) *Saccharomyces cerevisiae* has an inducible response to menadione which differs from that to hydrogen peroxide. *J Gen Microbiol* 139:501–507
- Francois J, Parrou JL (2001) Reserve carbohydrates metabolism in the yeast *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* 25:125–145
- Fuge EK, Braun EL, Werner-Washburne M (1994) Protein synthesis in long-term stationary-phase cultures of *Saccharomyces cerevisiae*. *J Bacteriol* 176:5802–5813
- Gachotte D, Eckstein J, Barbuch R, Hughes T, Roberts C, Bard M (2001) A novel gene conserved from yeast to humans is involved in sterol biosynthesis. *J Lipid Res* 42:150–154
- Garreau H, Hasan RN, Renault G, Estruch F, Boy-Marcotte E, Jacquet M (2000) Hyperphosphorylation of Msn2p and Msn4p in response to heat shock and the diauxic shift is inhibited by cAMP in *Saccharomyces cerevisiae*. *Microbiology* 146:2113–2120
- Gasch AP (2002) The environmental stress response: a common yeast response to environmental stresses. In: Hohmann S,

- Mager P (eds) Yeast stress responses. Springer, Berlin Heidelberg New York (in press)
- Gasch A, Spellman P, Kao C, Carmel-Harel O, Eisen M, Storz G, Botstein D, Brown P (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell* 11:4241–4257
- Gasch AP, Huang MX, Metzner S, Botstein D, Elledge SJ, Brown PO (2001) Genomic expression responses to DNA-damaging agents and the regulatory role of the yeast ATR homolog Mec1p. *Mol Biol Cell* 12:2987–3003
- Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon A, Cruciat C, Remor M, Hofert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier M, Copley R, Edelmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G, Superti-Furga G (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* 415:141–147
- Giaever G, Shoemaker DD, Jones TW, Liang H, Winzler EA, Astromoff A, Davis RW (1999) Genomic profiling of drug sensitivities via induced haploinsufficiency. *Nat Genet* 21:278–283
- Godon C, Lagniel G, Lee J, Buhler JM, Kieffer S, Perrot M, Boucherie H, Toledano MB, Labarre J (1998) The H₂O₂ stimulon in *Saccharomyces cerevisiae*. *J Biol Chem* 273:22480–22489
- Gorner W, Durchschlag E, Martinez-Pastor MT, Estruch F, Ammerer G, Hamilton B, Ruis H, Schuller C (1998) Nuclear localization of the C₂H₂ zinc finger protein Msn2p is regulated by stress and protein kinase A activity. *Genes Dev* 12:586–597
- Granot D, Snyder M (1993) Carbon source induces growth of stationary phase yeast cells, independent of carbon source metabolism. *Yeast* 9:465–479
- Gross C, Kelleher M, Iyer VR, Brown PO, Winge DR (2000) Identification of the copper regulon in *Saccharomyces cerevisiae* by DNA microarrays. *J Biol Chem* 275:32310–32316
- Haab BB, Dunham MJ, Brown PO (2001) Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. *Genome Biol* 2: 1–13
- Ho Y, Gruhler A, Heilbut A, Bader GD, Moore L, Adams SL, Millar A, Taylor P, Bennett K, Boutillier K, Yang L, Wolting C, Donaldson I, Schandorff S, Shewnarane J, Vo M, Taggart J, Goudreaux M, Muskut B, Alfarano C, Dewar D, Lin Z, Michalickova K, Willems AR, Sassi H, Nielsen PA, Rasmussen KJ, Andersen JR, Johansen LE, Hansen LH, Jespersen H, Podtelejnikov A, Nielsen E, Crawford J, Poulsen V, Sorensen BD, Matthiesen J, Hendrickson RC, Gleeson F, Pawson T, Moran MF, Durocher D, Mann M, Hogue CWV, Figeys D, Tyers M (2002) Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature* 415:180–183
- Hughes JD, Estep PW, Tavazoie S, Church GM (2000a) Computational identification of cis-regulatory elements associated with groups of functionally related genes in *Saccharomyces cerevisiae*. *J Mol Biol* 296:1205–1214
- Hughes TR, Marton MJ, Jones AR, Roberts CJ, Stoughton R, Armour CD, Bennett HA, Coffey E, Dai H, He YD, Kidd MJ, King AM, Meyer MR, Slade D, Lum PY, Stepaniants SB, Shoemaker DD, Gachotte D, Chakraborty K, Simon J, Bard M, Friend SH (2000b) Functional discovery via a compendium of expression profiles. *Cell* 102:109–126
- Igual JC, Estruch F (2000) Signalling stress in yeast. *Food Technol Biotechnol* 38:263–276
- Ito T, Chiba T, Ozawa R, Yoshida M, Hattori M, Sakaki Y (2001) A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc Natl Acad Sci U S A* 98:4569–4574
- Iyer VR, Horak CE, Scafe CS, Botstein D, Snyder M, Brown PO (2001) Genomic binding sites of the yeast cell-cycle transcription factors SBF and MBF. *Nature* 409:533–538
- Jelinsky SA, Samson LD (1999) Global response of *Saccharomyces cerevisiae* to an alkylating agent. *Proc Natl Acad Sci USA* 96:1486–1491
- Jelinsky SA, Estep P, Church GM, Samson LD (2000) Regulatory networks revealed by transcriptional profiling of damaged *Saccharomyces cerevisiae* cells: Rpn4 links base excision repair with proteasomes. *Mol Cell Biol* 20:8157–8167
- Jia MH, Larossa RA, Lee JM, Rafalski A, Deroose E, Gonye G, Xue Z (2000) Global expression profiling of yeast treated with an inhibitor of amino acid biosynthesis, sulfometuron methyl. *Physiol Genomics* 3:83–92
- Johnston M (1999) Feasting; fasting and fermenting: glucose sensing in yeast and other cells. *Trends Genet* 15:29–33
- Jung US, Levin DE (1999) Genome-wide analysis of gene expression regulated by the yeast cell wall integrity signalling pathway. *Mol Microbiol* 34:1049–1057
- Kapteyn JC, ter Riet B, Vink E, Blad S, De Nobel H, Van Den Ende H, Klis FM (2001) Low external pH induces HOG1-dependent changes in the organization of the *Saccharomyces cerevisiae* cell wall. *Mol Microbiol* 39:469–479
- Klein C, Struhl K (1994) Protein kinase A mediates growth-regulated expression of yeast ribosomal protein genes by modulating RAP1 transcriptional activity. *Mol Cell Biol* 14:1920–1928
- Kuhn KM, DeRisi JL, Brown PO, Sarnow P (2001) Global and specific translational regulation in the genomic response of *Saccharomyces cerevisiae* to a rapid transfer from a fermentable to a nonfermentable carbon source. *Mol Cell Biol* 21:916–927
- Kwast KE, Lai LC, Menda N, James DT 3rd, Aref S, Burke PV (2002) Genomic analyses of anaerobically induced genes in *Saccharomyces cerevisiae*: functional roles of Rox1 and other factors in mediating the anoxic response. *J Bacteriol* 184:250–265
- Lee J, Godon C, Lagniel G, Spector D, Garin J, Labarre J, Toledano MB (1999) Yap1 and Skn7 control two specialized oxidative stress response regulons in yeast. *J Biol Chem* 274:16040–16046
- Lee S, Pellicoli A, Demeter J, Vaze M, Gasch AP, Malkova A, Brown PO, Stearns T, Foiani M, Haber JE (2001) Arrest, adaptation and recovery following a chromosome double-strand break in *Saccharomyces cerevisiae*. *Cold Spring Harbor Symp Quant Biol* 65:303–314
- Lewis JG, Learmonth RP, Watson K (1995) Induction of heat, freezing and salt tolerance by heat and salt shock in *Saccharomyces cerevisiae*. *Microbiology* 141:687–694
- Li Y, Moir RD, Sathy-Coraci IK, Warner JR, Willis IM (2000) Repression of ribosome and tRNA synthesis in secretion-defective cells is signaled by a novel branch of the cell integrity pathway. *Mol Cell Biol* 20:3843–3851
- Lieb JD, Liu X, Botstein D, Brown PO (2001) Promoter-specific binding of Rap1 revealed by genome-wide maps of protein-DNA association. *Nat Genet* 28:327–334
- Liu PC, Thiele DJ (2001) Novel stress-responsive genes *EMG1* and *NOPI4* encode conserved, interacting proteins required for 40S ribosome biogenesis. *Mol Biol Cell* 12:3644–3657
- Lyons TJ, Gasch AP, Gaither LA, Botstein D, Brown PO, Eide DJ (2000) Genome-wide characterization of the Zap1p zinc-responsive regulon in yeast. *Proc Natl Acad Sci USA* 97:7957–7962
- Macfarlane WM, McKinnon CM, Felton-Edkins ZA, Cragg H, James RFL, Docherty K (1999) Glucose stimulates translocation of the homeodomain transcription factor PDX1 from the cytoplasm to the nucleus in pancreatic beta-cells. *J Biol Chem* 274:1011–1016
- Marchler G, Schuller C, Adam G, Ruis H (1993) A *Saccharomyces cerevisiae* UAS element controlled by protein kinase A activates transcription in response to a variety of stress conditions. *EMBO J* 12:1997–2003
- Mitchel RE, Morrison DP (1982) Heat-shock induction of ionizing radiation resistance in *Saccharomyces cerevisiae*, and correlation with stationary growth phase. *Radiat Res* 90:284–291
- Mizumoto K, Glascott PA Jr, Farber JL (1993) Roles for oxidative stress and poly(ADP-ribosylation) in the killing of cultured hepatocytes by methyl methanesulfonate. *Biochem Pharmacol* 46:1811–1818

- Momose Y, Iwahashi H (2001) Bioassay of cadmium using a DNA microarray: genome-wide expression patterns of *Saccharomyces cerevisiae* response to cadmium. *Environ Toxicol Chem* 20:2353–2360
- Natarajan K, Meyer MR, Jackson BM, Slade D, Roberts C, Hinnebusch AG, Marton MJ (2001) Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Mol Cell Biol* 21:4347–4368
- Nelson MA, Metzenberg RL (1992) Sexual development genes of *Neurospora crassa*. *Genetics* 132:149–162
- Neuman-Silberberg FS, Bhattacharya S, Broach JR (1995) Nutrient availability and the RAS/cyclic AMP pathway both induce expression of ribosomal protein genes in *Saccharomyces cerevisiae* but by different mechanisms. *Mol Cell Biol* 15:3187–3196
- Ni L, Snyder M (2001) A genomic study of the bipolar bud site selection pattern in *Saccharomyces cerevisiae*. *Mol Biol Cell* 12:2147–2170
- Nierras CR, Warner JR (1999) Protein kinase C enables the regulatory circuit that connects membrane synthesis to ribosome synthesis in *Saccharomyces cerevisiae*. *J Biol Chem* 274:13235–13241
- Norbeck J, Blomberg A (1996) Protein expression during exponential growth in 0.7 M NaCl medium of *Saccharomyces cerevisiae*. *FEMS Microbiol Lett* 137:1–8
- Norbeck J, Blomberg A (2000) The level of cAMP-dependent protein kinase A activity strongly affects osmotolerance and osmo-instigated gene expression changes in *Saccharomyces cerevisiae*. *Yeast* 16:121–137
- Ogawa N, DeRisi J, Brown PO (2000) New components of a system for phosphate accumulation and polyphosphate metabolism in *Saccharomyces cerevisiae* revealed by genomic expression analysis. *Mol Biol Cell* 11:4309–4321
- Ooi SL, Shoemaker DD, Boeke JD (2001) A DNA microarray-based genetic screen for nonhomologous end-joining mutants in *Saccharomyces cerevisiae*. *Science* 294:2552–2556
- Padilla PA, Fuge EK, Crawford ME, Errett A, Werner-Washburne M (1998) The highly conserved, coregulated *SNO* and *SNZ* gene families in *Saccharomyces cerevisiae* respond to nutrient limitation. *J Bacteriol* 180:5718–5726
- Paik WK, DiMaria P, Kim S, Magee PN, Lotlikar PD (1984) Alkylation of protein by methyl methanesulfonate and 1-methyl-1-nitrosourea in vitro. *Cancer Lett* 23:9–17
- Pan XW, Harashima T, Heitman J (2000) Signal transduction cascades regulating pseudohyphal differentiation of *Saccharomyces cerevisiae*. *Curr Opin Microbiol* 3:567–572
- Posas F, Chambers JR, Heyman JA, Hoeffler JP, de Nadal E, Arino J (2000) The transcriptional response of yeast to saline stress. *J Biol Chem* 275:17249–17255
- Powers T, Walter P (1999) Regulation of ribosome biogenesis by the rapamycin-sensitive TOR- signaling pathway in *Saccharomyces cerevisiae*. *Mol Biol Cell* 10:987–1000
- Primig M, Williams RM, Winzeler EA, Tevzadze GG, Conway AR, Hwang SY, Davis RW, Esposito RE (2000) The core meiotic transcriptome in budding yeasts. *Nat Genet* 26:415–423
- Rabitsch KP, Toth A, Galova M, Schleiffer A, Schaffner G, Aigner E, Rupp C, Penkner AM, Moreno-Borchart AC, Primig M, Esposito RE, Klein F, Knop M, Nasmyth K (2001) A screen for genes required for meiosis and spore formation based on whole-genome expression. *Curr Biol* 11:1001–1009
- Ren B, Robert F, Wyrick JJ, Aparicio O, Jennings EG, Simon I, Zeitlinger J, Schreiber J, Hannett N, Kanin E, Volkert TL, Wilson CJ, Bell SP, Young RA (2000) Genome-wide location and function of DNA binding proteins. *Science* 290:2306–2309
- Rep M, Reiser V, Gartner U, Thevelein JM, Hohmann S, Ammerer G, Ruis H (1999) Osmotic stress-induced gene expression in *Saccharomyces cerevisiae* requires Msn1p and the novel nuclear factor Hot1p. *Mol Cell Biol* 19:5474–5485
- Rep M, Krantz M, Thevelein JM, Hohmann S (2000) The transcriptional response of *Saccharomyces cerevisiae* to osmotic shock. Hot1p and Msn2p/Msn4p are required for the induction of subsets of high osmolarity glycerol pathway-dependent genes. *J Biol Chem* 275:8290–8300
- Roberts CJ, Nelson B, Marton MJ, Stoughton R, Meyer MR, Bennett HA, He YD, Dai H, Walker WL, Hughes TR, Tyers M, Boone C, Friend SH (2000) Signaling and circuitry of multiple MAPK pathways revealed by a matrix of global gene expression profiles. *Science* 287:873–880
- Ross-Macdonald P, Coelho PSR, Snyder M (1999) Large-scale analysis of the yeast genome by transposon tagging and gene disruption. *Nature* 402:413–418
- Schüller C, Brewster JL, Alexander MR, Gustin MC, Ruis H (1994) The HOG pathway controls osmotic regulation of transcription via the stress response element (STRE) of the *Saccharomyces cerevisiae* *CTT1* gene. *EMBO* 13:4382–4389
- Searfoss AM, Wickner RB (2000) 3' poly(A) is dispensable for translation. *Proc Natl Acad Sci USA* 97:9133–9137
- Siderius M, Rots E, Mager WH (1997) High-osmolarity signalling in *Saccharomyces cerevisiae* is modulated in a carbon-source-dependent fashion. *Microbiology* 143:3241–3250
- Siderius M, VanWuytswinkel O, Reijenga KA, Kelders M, Mager WH (2000) The control of intracellular glycerol in *Saccharomyces cerevisiae* influences osmotic stress response and resistance to increased temperature. *Mol Microbiol* 36:1381–1390
- Simon I, Barnett J, Hannett N, Harbison CT, Rinaldi NJ, Volkert TL, Wyrick JJ, Zeitlinger J, Gifford DK, Jaakkola TS, Young RA (2001) Serial regulation of transcriptional regulators in the yeast cell cycle. *Cell* 106:697–708
- Sogin SJ, Saunders CA (1980) Fluctuation in polyadenylate size and content in exponential and stationary-phase cells of *Saccharomyces cerevisiae*. *J Bacteriol* 144:74–81
- Spellman PT, Sherlock G, Zhang MQ, Iyer VR, Anders K, Eisen MB, Brown PO, Botstein D, Futcher B (1998) Comprehensive identification of cell-cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol Biol Cell* 9:3273–3297
- Sumner ER, Avery SV (2002) Phenotypic heterogeneity: differential stress resistance among individual cells of the yeast *Saccharomyces cerevisiae*. *Microbiology* 148:345–51
- Takizawa PA, DeRisi JL, Wilhelm JE, Vale RD (2000) Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. *Science* 290:341–344
- Tavazoie S, Hughes JD, Campbell MJ, Cho RJ, Church GM (1999) Systematic determination of genetic network architecture. *Nat Genet* 22:281–285
- Thevelein JM, de Winde JH (1999) Novel sensing mechanisms and targets for the cAMP-protein kinase A pathway in the yeast *Saccharomyces cerevisiae*. *Mol Microbiol* 33:904–918
- Thevelein JM, Cauwenberg L, Colombo S, DeWinde JH, Donation M, Dumortier F, Kraakman L, Lemaire K, Ma P, Nauwelaers D, Rolland F, Teunissen A, VanDijck P, Versele M, Wera S, Winderickx J (2000) Nutrient-induced signal transduction through the protein kinase A pathway and its role in the control of metabolism; stress resistance; and growth in yeast. *Enzyme Microb Technol* 26:819–825
- Tong AH, Evangelista M, Parsons AB, Xu H, Bader GD, Page N, Robinson M, Raghibizadeh S, Hogue CW, Bussey H, Andrews B, Tyers M, Boone C (2001) Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science* 294:2364–2368
- Traven A, Wong JMS, Xu DM, Sopta M, Ingles CJ (2001) Interorganellar communication: altered nuclear gene expression profiles in a yeast mitochondrial DNA mutant. *J Biol Chem* 276:4020–4027
- Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P (2000) Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell* 101:249–258
- Treger JM, Schmitt AP, Simon JR, McEntee K (1998) Transcriptional factor mutations reveal regulatory complexities of heat shock and newly identified stress genes in *Saccharomyces cerevisiae*. *J Biol Chem* 273:26875–26879

- Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P, Qureshi-Emili A, Li Y, Godwin B, Conover D, Kalbfleisch T, Vijayadamodar G, Yang M, Johnston M, Fields S, Rothberg JM (2000) A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* 403:623–627
- Valencia M, Bentele M, Vaze MB, Herrmann G, Kraus E, Lee SE, Schar P, Haber JE (2001) NEJ1 controls non-homologous end joining in *Saccharomyces cerevisiae*. *Nature* 414:666–669
- Vasudevan S, Peltz SW (2001) Regulated ARE-mediated mRNA decay in *Saccharomyces cerevisiae*. *Mol Cell* 7:1191–1200
- Vincent O, Townley R, Kuchin S, Carlson M (2001) Subcellular localization of the Snf1 kinase is regulated by specific beta subunits and a novel glucose signaling mechanism. *Genes Dev* 15:1104–1114
- Wang P, Perfect JR, Heitman J (2000) The G-protein beta subunit GPB1 is required for mating and haploid fruiting in *Cryptococcus neoformans*. *Mol Cell Biol* 20:352–362
- Warner JR (1999) The economics of ribosome biosynthesis in yeast. *Trends Biochem Sci* 24:437–440
- Werner-Washburne M, Becker J, Kosc-Smithers J, Craig EA (1989) Yeast Hsp70 RNA levels vary in response to the physiological status of the cell. *J Bacteriol* 171:2680–2688
- Werner-Washburne M, Braun E, Johnston GC, Singer RA (1993) Stationary phase in the yeast *Saccharomyces cerevisiae*. *Microbiol Rev* 57:383–401
- Werner-Washburne M, Braun EL, Crawford ME, Peck VM (1996) Stationary phase in *Saccharomyces cerevisiae*. *Mol Microbiol* 19:1159–1166
- Wieser R, Adam G, Wagner A, Schuller C, Marchler G, Ruis H, Krawiec Z, Bilinski T (1991) Heat shock factor-independent heat control of transcription of the CTT1 gene encoding the cytosolic catalase T of *Saccharomyces cerevisiae*. *J Biol Chem* 266:12406–12411
- Wilhelm D, Bender K, Knebel A, Angel P (1997) The level of intracellular glutathione is a key regulator for the induction of stress-activated signal transduction pathways including Jun N-terminal protein kinases and p38 kinase by alkylating agents. *Mol Cell Biol* 17:4792–4800
- Winzler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H, Chu AM, Connolly C, Davis K, Dietrich F, Dow SW, Bakkoury ME, Foury F, Friend SH, Gentale E, Giaever G, Hegemann JH, Jones T, Laub M, Liao H, Liebundguth N, Lockhart DJ, LucauDanila A, Lussier M, Rabet NM, Menard P, Mittmann M, Pai C, Rebischung C, Revuelta JL, Riles L, Roberts CJ, RossMacDonald P, Scherens B, Snyder M, SookhaiMahadeo S, Storms RK, Veronneau S, Voet M, Volckaert G, Ward TR, Wysocki R, Yen GS, Yu KX, Zimmermann K, Philippsen P, Johnston M, Davis RW (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285:901–906
- Yale J, Bohnert HJ (2001) Transcript expression in *Saccharomyces cerevisiae* at high salinity. *J Biol Chem* 276:15996–16007
- Zaragoza O, Gancedo JM (2000) Pseudohyphal growth is induced in *Saccharomyces cerevisiae* by a combination of stress and cAMP signalling. *Antonie Van Leeuwenhoek Int J Gen Mol Microbiol* 78:187–194
- Zhu H, Snyder M (2001) Protein arrays and microarrays. *Curr Opin Chem Biol* 5:40–45
- Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, Bertone P, Lan N, Jansen R, Bidlingmaier S, Houfek T, Mitchell T, Miller P, Dean RA, Gerstein M, Snyder M (2001) Global analysis of protein activities using proteome chips. *Science* 293:2101–2105